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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,280,418**, on August 12, 1999, by **DONALD S. FORSYTH**, for "Micro Extraction Technique". The said invention was made while Donald S. Forsyth was employed as a public servant as defined in the Public Servants Inventions Act with the **Minister of Health**, and pursuant to Section 5 of that Act, the said invention has been determined to be vested in **Her Majesty the Queen in Right of Canada, as represented by the Minister of Health.**

Agent certificateur/Certifying Officer

May 4, 2004
Date

Canada

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04-09-02

OPIC CIPO

Micro Extraction Technique

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the method for solid phase micro extraction and analysis and in particular to the micro extraction and analysis using various types of solid support which can be coated with various materials.

2. Description of the Prior Art

The analysis of environmental, food, biomedical and pharmaceutical samples involves the separation of components or analytes of interest from the sample matrix such as soil, water, food, tissue or other material. Liquid/liquid extraction is a traditional method of sample extraction and separation- an example is the extraction of a water sample with an immiscible organic solvent such as hexane. Solid samples can be mixed with anhydrous sodium sulfate, ground to a free flowing powder, packed into a chromatography column and then extracted with an organic solvent. Solvent-based extraction methods are frequently time consuming and expensive since high purity solvents must be purchased and properly disposed. Also, many solvents are relatively toxic and should be handled only under proper laboratory conditions. Solvent extraction is not very selective so further analyte isolation is usually accomplished by other chromatographic techniques before the analytes can be quantitated. This increases the cost and complexity of the analysis.

Solid phase extraction is an alternative to liquid/liquid extraction which involves the selective adsorption of the analytes of interest onto a sorbent (typically a chemically modified silica surface) while other co extractives are unretained. Pre-made solid phase extraction cartridges are available but there is significant variation between manufacturers products. The analytes of interest are then eluted from the sorbent by the use of selective solvent or solvent mixtures. The analyte(s) can then be analyzed. Many different types of sorbents are available so that a wide range of selectivities are possible. Although solvent use is reduced relative to liquid/liquid extraction, purchase and disposal costs remain and the procedure is still time consuming.

Solid phase micro extraction consists of two processes- partitioning of analytes between the coating (sorbent) and sample and the desorption of the collected analytes into an analytical instrument. This is accomplished by exposing the coated fibre to the sample, allowing the target analytes to be adsorbed into the coating from either the head space above the sample or from the sample directly. Various coatings are available to adjust selectivity of the extraction and no solvent is used for the extraction. After exposure, the fibre (now containing concentrated analytes) is then typically thermally desorbed in an instrument followed by separation and quantitation of the analytes. The method is simple, inexpensive and eliminates some of the disadvantages of solid phase extraction including: solvent consumption, extract preconcentration and high blank values. However, solid phase micro extraction has a significant limitation of low sample throughput which has been only partially overcome by expensive automation options.

SUMMARY OF THE INVENTION

A technique for carrying out solid phase micro extraction of analytes contained within a liquid, solid or other material is characterized by, in combination, a fibre assembly or assemblies and a gas tight sampling enclosure. The fibre assembly or assemblies are mounted such that it/they either sample the head space above the sample or sample the sample matrix directly within the gas tight enclosure. The technique is characterized by the steps of choosing either/or the fibre and coating for the fibre assembly based on selectivity of the fibre and/or coating for at least one of the analytes present in the sample. The technique is further characterized by exposing said fibre or fibre assemblies a sufficient time to permit chemical extraction to occur, ending said contact and then placing said fibre(s) into a suitable micro volume of solvent where desorption of the analytes from the fibre(s) occur. Quantitation of the analytes in the extracting solvent can then proceed with a suitable analytical instrumentation.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a an a) schematic side view and b) enlarged end view of a fibre assembly.

Fig. 1b a schematic side view of a shielded fibre assembly.

Fig. 2 a schematic side view of a fibre assembly installed in a typical sampling enclosure.

Fig. 3 the reproducibility of multiple extractions using the present technique.

Fig. 4 a chromatogram of the extraction of a chlorinated pesticide mixture containing 1) gamma-BHC (lindane), 2) heptachlor, 3) aldrin, 4) dieldrin, 5) endrin and 6) 4,4'-DDT from an aqueous sample.

Fig. 5 a chromatogram of the extraction of a BTEX mixture containing 1) benzene, 2) toluene, 3) ethylbenzene, 4) *p*-xylene, 5) *m*-xylene and 6) *o*-xylene from an aqueous sample.

DESCRIPTION OF A PREFERRED EMBODIMENT

Referring to Fig. 1a in greater detail, a fibre assembly for carrying out solid phase micro extraction has a cylindrical support 1 which may have a length of coating 2 of which various types could be used. The diameter of the fibre assembly may vary but would generally be between 0.5 to 2 mm and the cylindrical support may be solid or hollow. Alternatively, in Fig 1b a cylindrical support 1 which has a length of coating is mounted through a teflon faced septa 4; stainless steel tubing 3 of slightly longer length than the coating is mounted over the fibre assembly. In Fig 2., the fibre assembly 1 is mounted in a gas tight enclosure 2, typically but not exclusively a hypo-vial (trade mark) with an open top crimp top (as illustrated) 3 or screw cap and Teflon-faced seal 4. Generally, a Teflon coated magnetic stir bar 5 is enclosed to permit agitation of the sample 6 during collection of the analytes

onto the fibre assembly 1. The fibre assembly in Fig. 2 is positioned for head space sampling but can also used for direct (aqueous layer) sampling as well. Further, fibre assemblies can be manufactured with various coating lengths but 10 mm is the typical length used. Fibre assemblies can be constructed with fused silica or other support material chemically or mechanically modified followed by chemical attachment of the desired coating. Or, fibre assemblies can be constructed from silicone or other polymer tubing lengths, swelled by solvent and then dried (to a tight fit) over metal wire or other cylindrical support material. Polymer tubing used for fibre assembles is exhaustively extracted by solvent to virtually eliminate background levels of extractives prior to use. Fibre assemblies are typically heated to 100 C for 30 min before use to eliminate any volatiles that may have absorbed onto the fibre during storage of the fibre assembly.

The micro extraction technique consists of several simple steps. For example, when a water sample is to be analyzed for certain analytes, it is placed in a hypo-vial (trade-mark) or other suitable gas tight enclosure. The wire or other support material of the fibre assembly is inserted through the Teflon face of the vial seal into the silicone backing. If a shielded fibre assembly (Fig. 1b) is used, the support material extends through the vial seal . Single or multiple fibre assemblies can be mounted with identical or different coatings. The vial is then made gas tight by crimping the seal into position- other sealing techniques such as screw cap enclosures can be used. Preferably, the sample is stirred by a magnetic stir bar which was enclosed with the sample prior to sealing the vial. The sample may also be heated during extraction. The time required for extraction depends on many factors including the analytes being extracted as well as the thickness and type, if any, of coating on the fibre assembly. However, extraction times would normally range from several to 60 minutes. The vial is then opened and the analytes contained in the fibre assembly desorbed by immersion in a micro volume of solvent (typically 150 μL) contained in a small conical shaped vial such as a gas chromatograph autosampler vial. The fibre assembly can remain in the autosampler vial. If a shielded fibre assembly is used (Fig. 1b), the coating is retracted into the stainless steel tubing by pulling the cylindrical support (1, Fig. 1b) before opening the vial. The fibre assembly is then extended out from the shield into a micro volume of solvent. The extracted analytes are analyzed by injecting portions of the solvent extract. Various injection ports would be suited, including " programmable temperature", "split-splitless", "on-column" and "large solvent volume" types.

Careful construction and the typical one- time use of the fibre assemblies allows low variation of extraction efficiencies amongst fibre assemblies- Fig. 3 illustrates that relative standard deviations of extraction for four analytes in two trials of five individual extractions ranged from only 3.5 to 8.8%. These results, which are typical for the micro extraction technique, are comparable or superior to other analytical techniques. This consistency enables multiple simultaneous extractions to be conducted without costly automation, greatly enhancing sample throughput and quantitation. Further, since the analytes are desorbed into solvent, the extracts can be easily stored or collected until a convenient time for analysis. Solid phase micro extraction, however, is usually a sequential technique requiring the same device to be used repeatedly- this reduces sample throughput and analysis normally proceeds immediately after sampling. Also, since the analytes are usually thermally desorbed, there is no extract to archive or analyse by alternative analytical instrumentation.

The extraction process of the micro extraction technique is essentially that which occurs with solid phase micro extraction. The simple geometry of the fibre assembly resists clogging from particulates which may be present in the sample matrix. Further, extraction is usually not exhaustive but rather an equilibrium described by the partition coefficient between the water and organic stationary phase for the analytes. Selectivity of the technique can be altered by the appropriate choice of stationary phase for the analytes of interest. The partitioning between the aqueous phase and the

organic coating is described by the distribution constant, K:

$$K = C_f/C_{aq} \quad (1)$$

where C_f is the concentration in the stationary phase (coating) and C_{aq} is the concentration present in the water. Further, assuming a liquid polymeric coating, equation 2 shows that the amount of analyte absorbed by the coating at equilibrium can be related directly to its concentration in the sample

$$n=(K_{fs}V_fC_oV_s)/(K_{fs}V_f+V_s) \quad (2)$$

where n is the mass of the analyte absorbed by the coating, V_f and V_s are the volumes of the coating and sample respectively, K_{fs} is the partition coefficient between the coating and the sample matrix, and C_o is the initial concentration of the analyte in the sample. However, headspace solid phase micro extraction is a three-phase system (equation 3) where n can be expressed as:

$$n=(K_{fh}K_{hs}V_fC_oV_s)/(K_{fh}K_{hs}V_f+K_{hs}V_h+V_s) \quad (3)$$

when K_{fh} is the coating/gas distribution constant, K_{hs} is the gas/sample matrix constant and V_h is the volume of the headspace. A linear relationship therefore exists between the amount of analytes absorbed by the coating on the fibre assembly and the initial concentration of these analytes in the sample.

The limit of quantitation depends on the partition coefficient and the thickness of the coating and can extend down to sub part per billion. The rate of the extraction process is essentially the same as for solid phase micro extraction, where initially, the amount of analyte adsorbed by the coating (stationary phase) increases with increased extraction times until a point of steady state is achieved where the amount of analyte adsorbed remains relatively constant. At this point a state of equilibrium exists between the concentration of the analyte in the coating, headspace (if present) and in the sample matrix.

The micro extraction technique is very versatile and can be used for a variety of analytes. Figure 4 shows a chromatogram of a chlorinated pesticide mixture extracted and analyzed from water by the micro extraction technique with a unshielded silicone coated fibre assembly. The vapour pressure of these compounds range down to 1.5×10^{-7} mm Hg at room temperature, demonstrating that even a relatively nonvolatile compound can be readily quantitated by this technique. A gas chromatograph equipped with an electron capture detector was used for the analysis. Figure 5 shows the chromatogram of a BTEX mixture in water extracted and analysed by the micro extraction technique using a unshielded silicone coated fibre assembly. All of the components are present and readily quantitated by FID detector, illustrating that significant sample preconcentration is possible with micro solvent volume desorption of the fibres.

The micro extraction technique can be applied to any analysis suited to solid phase micro extraction since it can be transported for remote sampling, is amenable to existing analytical instrumentation and is quite flexible in terms of coatings. The ability to use multiple fibre assemblies would permit different coating chemistries to simultaneously extract a single sample. Further, since multiple samples can be extracted simultaneously, sample throughput is very high, permitting faster calibration and quantitation of samples. Possible fibre coatings include: carbowax (trade mark), silicone, polyimide, divinylbenzene, polyacrylate, carbon-based sorbents, ion-exchange materials and other materials readily apparent to skilled chemical analysts. The micro extraction technique can be

used with many different analytical methods, including gas, liquid or supercritical fluid chromatography, atomic absorption or emission, mass spectrometry, and infrared absorption spectrometry.

The method can be used for analysing not only environmental, biomedical and pharmaceutical samples but also industrial process streams, chemical reactions, and air monitoring. Further applications will be readily apparent to those skilled in the art.

CLAIMS

1. A microextraction device consisting of
   a support member having a coating thereon, the coating of a material capable of adsorbing or absorbing a desired analyte from a liquid phase when the support member with said coating is immersed in said liquid phase.
2. The device according to claim 1 wherein said support member is of a material chemically inert relative to the liquid phase.

3. A microextraction method comprising the steps of:
   immersing a microextraction device of claim 1 in a liquid phase,
   retaining the device in said liquid phase for a time sufficient for a n analyte to be adsorbed or absorbed in the coating of the device,
   removing the device from said liquid phase,
   desorbing said analyte from said coating into a volume of a solvent, and
   analysing said analyte from said solvent.

4. The method according to claim 3 wherein the step of immersing the device in a liquid phase is carried out in a gas-tight container.

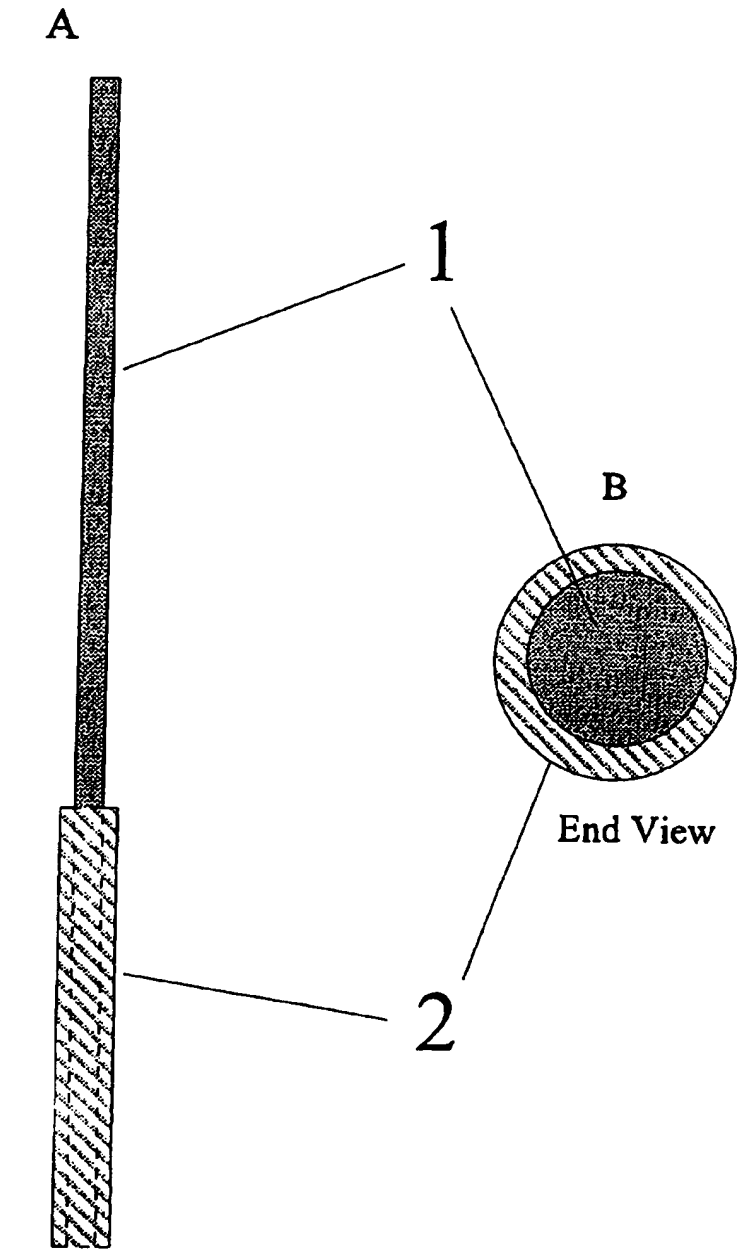


Figure 1a

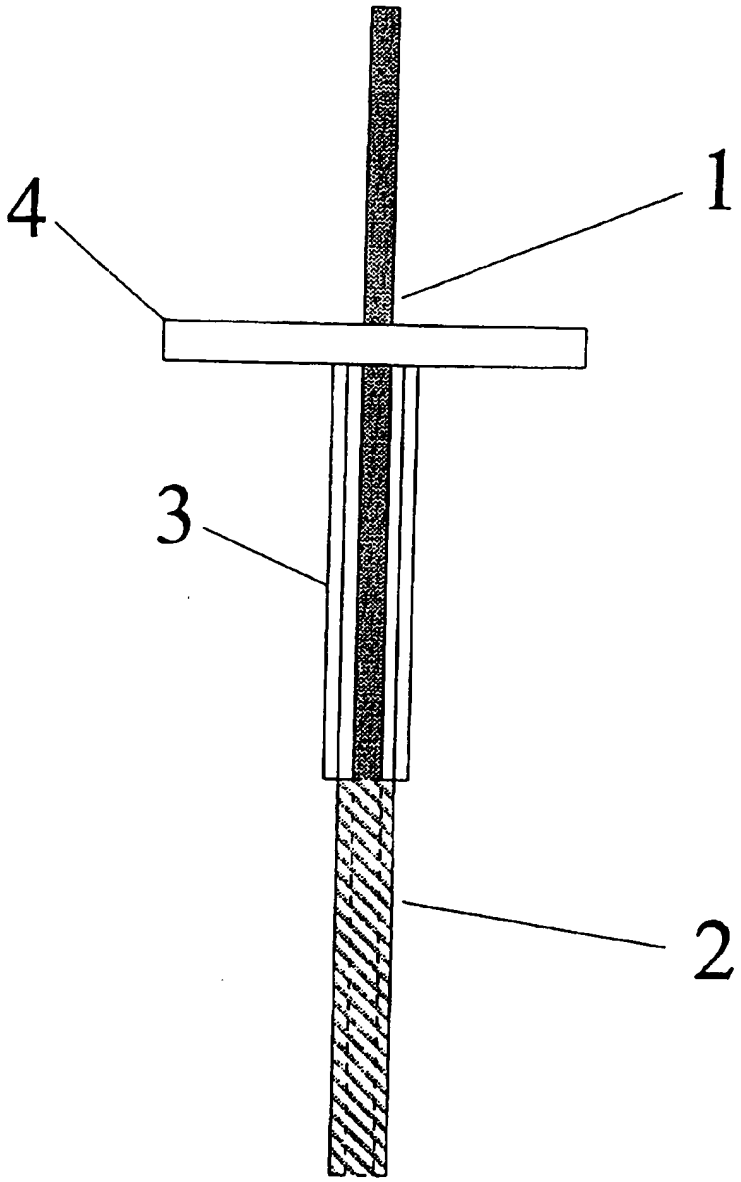


Figure 1b

Side View

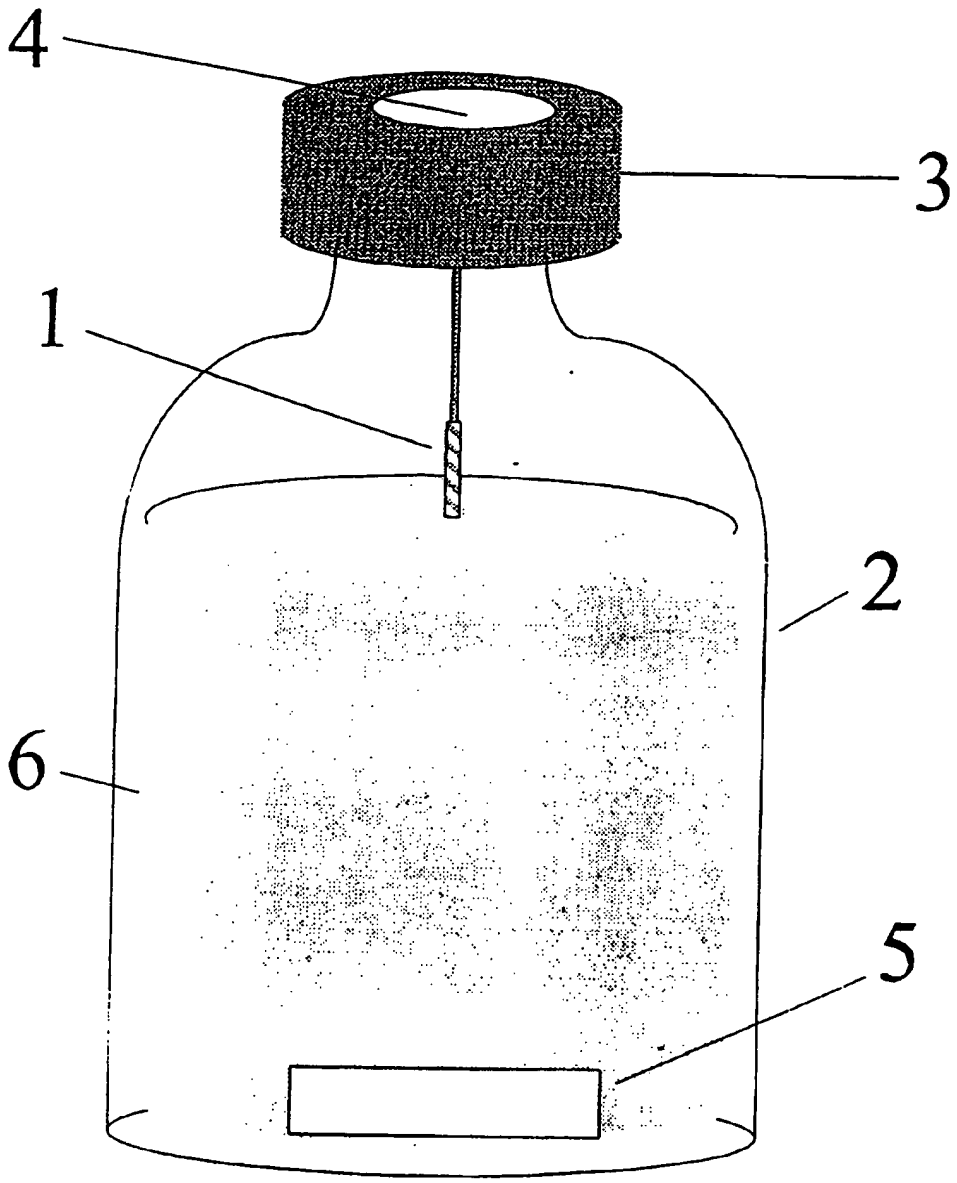


Figure 2

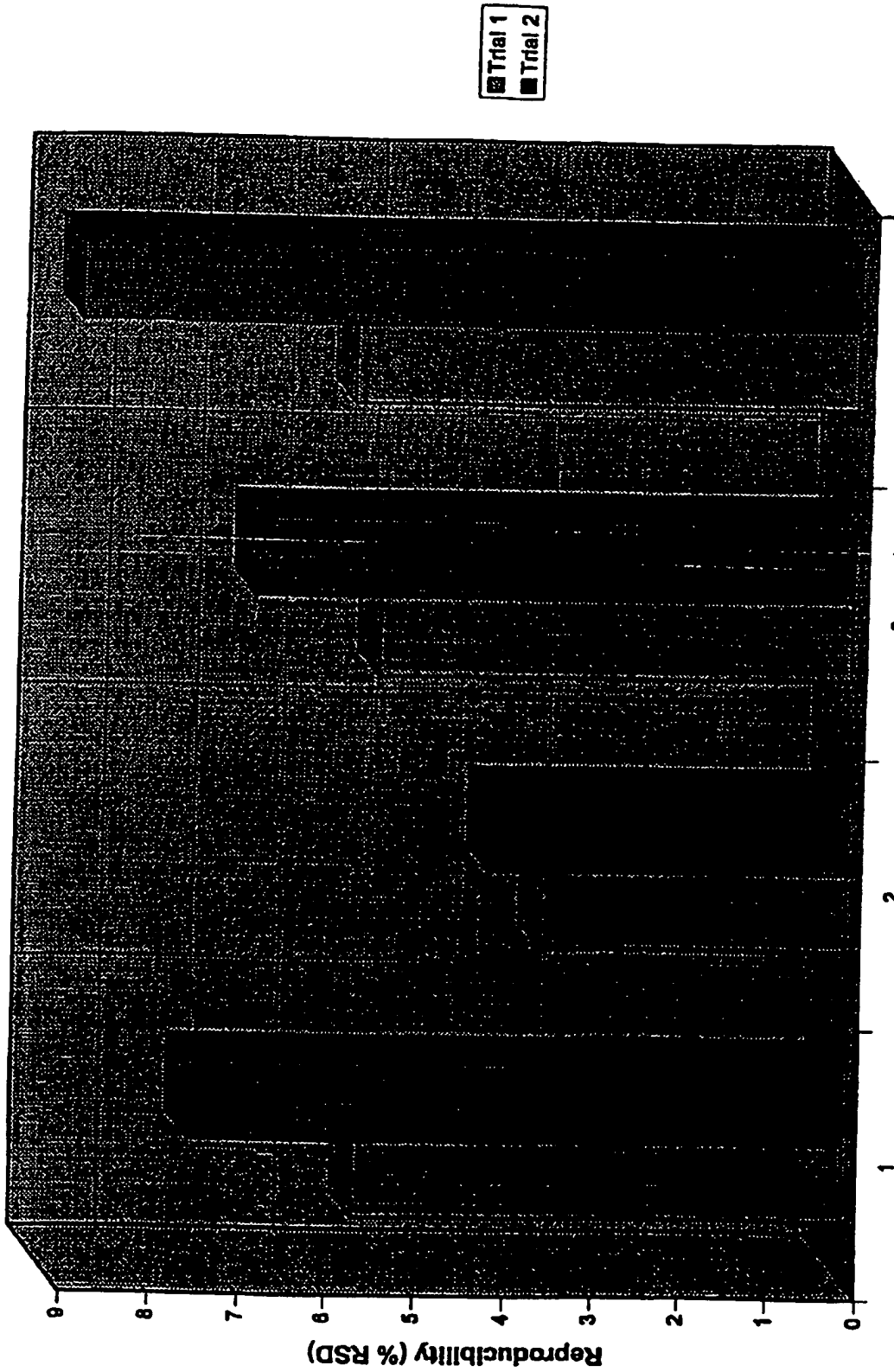
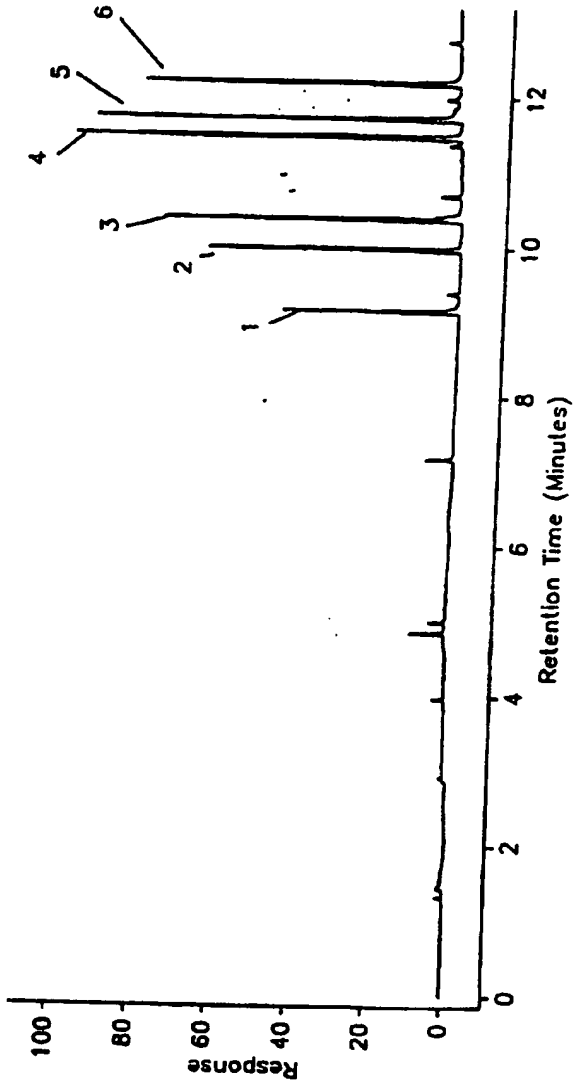


Figure 3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40
20
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2
4
6
8
10
12
Retention Time (Minutes)
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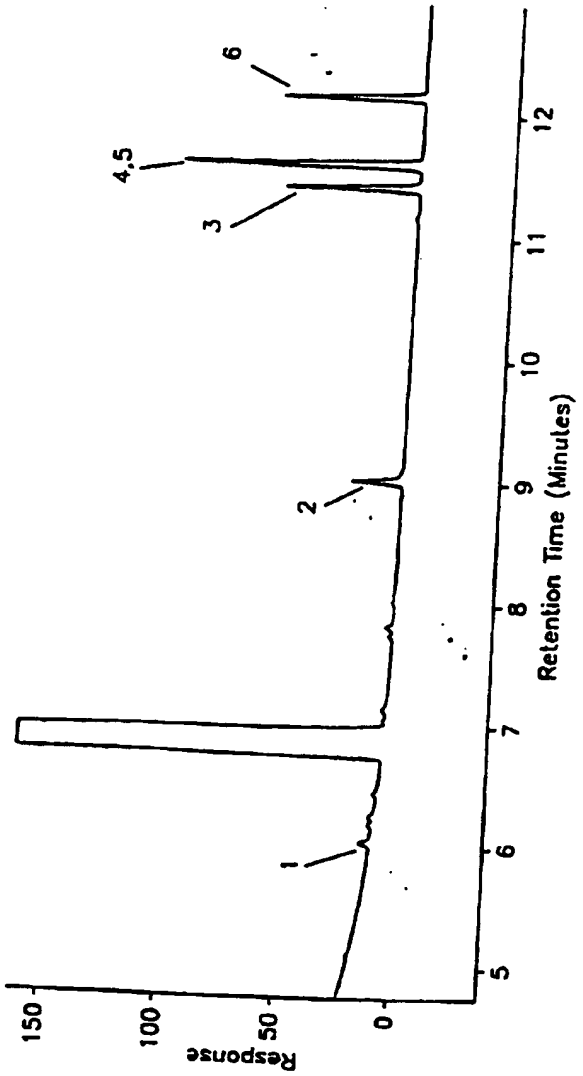
2
3
4
5
6

Figure 4


Response
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0
5
6
7
8
9
10
11
12
Retention Time (Minutes)
1
2
3
4,5
6

Figure 5